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Fecal carriage and antibiotic resistance of *Escherichia coli*, *Salmonella spp*. and *Enterococcus faecalis*. in day-old chicks imported into Benin.

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Background

Benin relies heavily on imports of day-old broiler chickens due to limited local hatchery capacity, sourcing them via formal (primarily European air transport) and informal (primarily Nigerian road transport) channels. Given the extent of the phenomenon of antibiotic resistance in the poultry industry in Benin, it was important to explore the role of the importation of day-old chicks and hatching eggs in the introduction of this resistance into poultry farms in Benin.

This study aims to assess the presence and resistance profiles of *Escherichia coli*, *Salmonella spp*., and *Enterococcus spp.* in imported day-old chicks in Benin.

Methods and Materials

Fecal samples were collected from day-old chick transport cartons. In each shipment we took 1 sample per batch of 1,000 chicks. After each collection, the samples were tested for the following bacteria: *Escherichia coli, Salmonella* spp*, Enterococcus feacalis* and *Enterococcus faecium.*

- *Escherichia coli* isolates were cultured on Chrom orientation medium and identified using the API 20E gallery.
- *Salmonella* spp. were pre-enriched with Rappaport-Vassiliadis soya broth, isolated on XLD medium, and identified using the API 20E gallery. Serogroup confirmation was done using specific serum agglutination.
- Enterococci were isolated on Slanetz-Bartley medium, and identification of *Enterococcus fecalis* and *Enterococcus faecium* was done by PCR targeting the tuf, ddl, and SodAEf genes.

Antibiotic sensitivity tests were carried out by disk diffusion according to EUCAST standards.

Results

200 day-old chick fecal samples were collected: 38 samples from Belgium, 58 from the Netherlands, and 104 from Nigeria (Figure 1). Frequencies of bacterial isolation are presented in Table 1. All 15 *Salmonella spp*. strains and all 13 strains of *Enterococcus fecalis* were isolated from Nigerian samples. Antibiotic resistance analysis is displayed in Figure 2. Medium levels of resistance to trimethoprim (42/144 for *Escherichia coli* and 3/15 for *Salmonella spp*) and tetracycline (74/144 for *Escherichia coli*), while Gram-negative bacilli exhibited low resistance to 3rd generation cephalosporins (10/144 for Escherichia *coli*). No resistance was noted for imipenem. No resistance to vancomycin was observed in enterococci. Resistance to tetracycline antibiotics, frequently used in poultry production, was more frequent for strains of *Escherichia coli* (52,38%) compared to strains of *Salmonella* spp (26,6%).

Figure 1. Distribution of samples according to origin

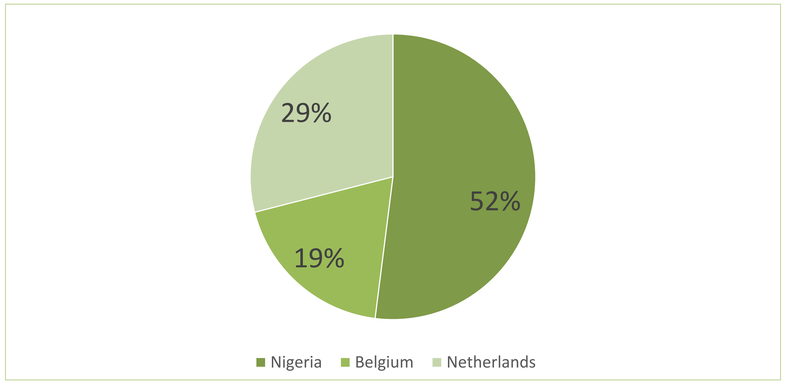
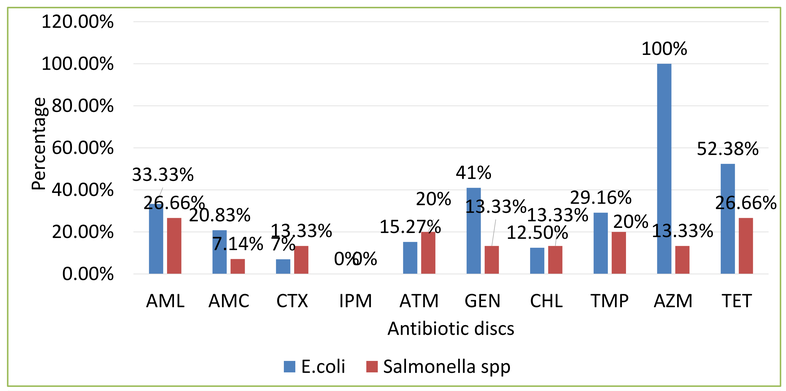


Table 1. Presentation of bacterial identification results according to origin.

	Escherichia coli	*Salmonella spp.*	*Enterococcus faecalis*
Nigeria	58/104 (55,77%)	15/104 (14,42%)	13/104 (12,50%)
Belgium	30/38 (78,94%)	0/38 (00%)	0/38 (00%)
Netherlands	56/58 (96,55%)	0/38 (00%)	0/38 (00%)
Total	144/200 (72%)	15/200 (7,5%)	13/200 (6,5%)

Figure 2. Percentage of antibiotic resistance of *Escherichia coli* and *Salmonella* spp. strains



AML: Amoxicillin; AMC: Amoxicillin + Clavulanic Acid; CTX: Cefotaxime; IMP: Imipenem; ATM: Aztreonam; GEN: Gentamycin; CHL: Chloramphenicol; TMP: Trimetroprime; AZM: Azithromycin; TET: Tetracycline.

Discussion

The significant presence of resistant strains particularly in informal circuits in Nigeria, poses crucial questions on the management of antibiotics in poultry farming.

These geographical variations can be attributed to differences in breeding practices and sanitary conditions depending on the country. It is also important to note that the hatcheries generally supplying day-old chicks through the informal sector are non-regulated hatcheries in Nigeria and therefore without control. These results agree with those of Rousham *et al.* (2018), Muloi *et al.* (2019).

The proportions of antibiotic resistance show that resistance is more noted for antibiotics often used in animal health and a low rate has been noted for antibiotics used in human health such as resistance of *Escherichia coli* to 3rd generation cephalosporins and carbapenems.

Conclusions

The study confirms the importation of day-old chicks as a potential source of antibiotic resistance in Benin, particularly from Nigeria. These results underline the importance of continuous monitoring of bacterial resistance at the borders of Benin, particularly in the informal entry circuits for day-old chicks. High resistance to some commonly used antibiotics, such as trimethoprim, gentamicin and tetracyclin, highlights the need to adopt stricter measures to limit the entry of these chicks into Benin or to promote more responsible management practices in order to prevent the spread of resistant strains once entering Beninese territory. Strict regulations must also be put in place to decide which pathogens are not acceptable in Beninese territory, also taking into account their resistance profiles. This study must be continued in order to produce more results for political decision-makers to better clean up the importation of day-old chicks into Benin.

Public Health Implications

The increase in antibiotic resistance among pathogenic bacteria originating from poultry farming poses considerable challenges for public health, although the specific resistances observed in this study show a low rate for antibiotics critical in human medicine. Strains of bacteria isolated from day-old chicks, although presenting low resistance to antibiotics used in human health, can still act as reservoirs of resistance genes. Poultry products contaminated with resistant bacteria can enter the human food chain, increasing the risk of spreading resistance genes. Additionally, poultry feces containing resistant bacteria can contaminate the environment, including soils and water sources, leading to indirect human exposure and increasing the chances of resistance spreading.

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